PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 17–25 (1980)

Long-Term Effects of Neonatal Administration of Estrogen and Progesterone, Alone or in Combination, on Male BALB/c and BALB/cfC3H Mice (40927)

LOVELL A. JONES[1]

Department of Zoology and Cancer Research Laboratory, University of California, Berkeley, California 94720

Abstract. Newborn BALB/c and BALB/cfC3H male mice were given five daily injections of 17β-estradiol and progesterone, alone and in combination, beginning within 36 hr after birth. Mice were killed and autopsied between 19 and 27 months of age. Mice treated neonatally with 17β-estradiol showed a complete inhibition of spermatogenesis in 71% of the BALB/c substrain (compared with 30% in the controls) and in 62% of the BALB/cfC3H substrain (compared with 0% in the controls), with 8 and 10 animals, respectively, having at least one testis completely atrophied. Estradiol alone resulted in epididymal cysts and some metaplastic changes in the secondary sex accessories in both substrains. Estradiol/progesterone combinations resulted in similar abnormalities in the male genital tract of both substrains, whereas progesterone alone was not so effective.

Abnormalities of the human reproductive system have been shown to occur in daughters of mothers who received synthetic sex hormones, especially diethylstilbestrol (DES), antenatally (1). Mice exposed antenatally or postnatally to steroid sex hormones and DES also develop abnormalities of the female genital tract (2, 3). Recent reports have described the possible consequences to human male offspring of intrauterine exposure to DES (1). So far no cancerous changes have been observed in the males, but possible infertility due to pathologic changes in the genital tract has been reported (1, 4, 5).

In male rats, Arai (6–8) reported that injections of estrogen for 30 days from the day of birth brought about an inhibition of spermatogenesis and a marked atrophy of reproductive accessories (see also 9). Dunn and Green (9) found that mice receiving a single injection of DES on the day of birth developed epididymal cysts and changes in the testis by one year of age. The effects of neonatal estradiol treatment on male mice have also been reported (10–13) including hyperplastic and metaplastic changes in the epithelium of the epididymis, the seminal vesicle and the ventral prostate, and inhibition of spermatogenesis. Neonatal DES administration to rats and antenatal DES administration to mice and hamsters also result in genital tract changes in males, including sterility (14–16). Two cases of invasive squamous-cell carcinoma of the genital tract of neonatally DES-treated castrated male rats have been recently reported (17). The present study examines the long-term effects of neonatal progesterone, alone and in combination with estradiol, on the genital tract of male mice.

Materials and methods. Totals of 97 BALB/c and 109 BALB/cfC3H newborn male mice survived to autopsy. Mice of each substrain were separated into six groups (Table I). Members of newborn litters were randomly distributed among several groups. Experimental mice received sc injections of 5 or 20 μg of 17β-estradiol; or 100 μg of progesterone; or 5 μg of 17β-estradiol + 100 μg of progesterone; or 20 μg of 17β-estradiol + 100 μg of progesterone, in 0.02 ml of sesame oil (the vehicle) for 5 days beginning within 36 hr after birth. The mice were weaned at 25 days of age.

Mice were killed and autopsied between 19 and 27 months of age; the mean age of controls was 23.4 months for BALB/c mice and 24.8 months for BALB/cfC3H. The mean age of experimental BALB/c mice was 24.4 months and of BALB/cfC3H mice

[1] Present address: Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California 94143.

0037-9727/80/090017-09$01.00/0

TABLE I. TESTICULAR DEVELOPMENT IN OLD NEONATALLY STEROID-TREATED MALE BALB/C AND BALB/CFC3H MICE

Neonatal treatment (daily dose in μg)		Strain	No. of mice	Age (months)	Body wt (g)	Degree of spermatogenesis*					No. of animals with one or both testes replaced by fibrous tissue
Estradiol	Progesterone					–	±	+	++	+++	++++	
—	—	BALB/c	10	23.5 ± 5.1	29.9 ± 5.1	3	1	3	3	0	0	0
—	—	BALB/cfC3H	13	24.8 ± 4.6	29.2 ± 4.6	0	3	3	1	3	3	0
5	—	BALB/c	14	24.0 ± 1.4	30.0 ± 3.4	9	3	1	1	0	0	4
5	—	BALB/cfC3H	12	23.9 ± 2.0	29.0 ± 2.8	5	4	2	1	0	0[a]	1
20	—	BALB/c	7	24.4 ± 0.9	23.5 ± 2.6	6	1	0	0	0	0	4
20	—	BALB/cfC3H	17	25.0 ± 3.1	25.0 ± 3.1	13	2	2	0	0	0[b]	9
—	100	BALB/c	22	24.0 ± 1.2	28.8 ± 4.0	4	3	8	7	0	0	1
—	100	BALB/cfC3H	12	23.7 ± 1.3	30.6 ± 2.9	0	0	6	3	3	0[b]	0
5	100	BALB/c	17	23.9 ± 2.1	26.7 ± 3.8	9	3	3	1	1	0	2
5	100	BALB/cfC3H	22	23.7 ± 1.5	28.6 ± 3.6	7	10	3	1	1	0[b]	2
20	100	BALB/c	27	25.2 ± 1.1	26.2 ± 3.0	16	5	3	2	1	0	9
20	100	BALB/cfC3H	31	24.4 ± 3.4	26.5 ± 3.4	16	7	6	2	0	0[b]	4

* See text for explanation of grading.
[a] Differs from untreated BALB/cfC3H control, $P < 0.05$.
[b] Differs from untreated BALB/cfC3H control, $P < 0.005$.

23.9 months. Testes, epididymides, ventral and dorsal prostates, seminal vesicles, coagulating glands, ducti deferentes, and adrenal glands were fixed in Bouin's fluid and sectioned in paraffin at a thickness of 7 μm; sample sections were stained with Harris' hematoxylin and eosin.

Spermatogenetic activity was graded by counting the number of seminiferous tubule sections containing spermatozoa and dividing that number by the total number of seminiferous tubules present. In Table I, – = ≤10%, + = ≤25%, ++ = ≤50%, +++ = ≤75%, and ++++ = >75%. The determination was done on a midsagittal section of one testis from each animal. Data were statistically analyzed using chi-square or Fisher's exact test and the variance test for homogeneity of binomial distribution.

Results. Body weight. The average body weight of the neonatally hormone-treated groups was not significantly different from that of the control groups, with the exception of those groups treated with 20 μg of 17β-estradiol alone, where the body weights were significantly lower than those of the respective control mice of each substrain ($P < 0.05$).

Testes. The testes of three 2-year-old BALB/c control mice lacked spermatozoa and the cells lining the seminiferous tubules showed many vacuoles (Fig. 1). However, no testes from either BALB/c or BALB/cfC3H control mice were completely degenerated, and in most cases the testes were generally normal in appearance (Fig. 2). By inspection all experimental groups with estradiol appeared to have an increased proportion with – or ± as compared to controls (Table I). None of the individual comparisons of neonatally estradiol or estradiol/progesterone-treated BALB/c mice with controls were significantly different by chi-square or Fisher's exact test. However, spermatogenesis was significantly reduced in all experimental groups treated neonatally with estradiol when compared with the controls ($0.025 > P > 0.01$). Comparisons of spermatogenesis in each experimental BALB/cfC3H group with controls was significantly different (Table I). Neonatal progesterone treatment

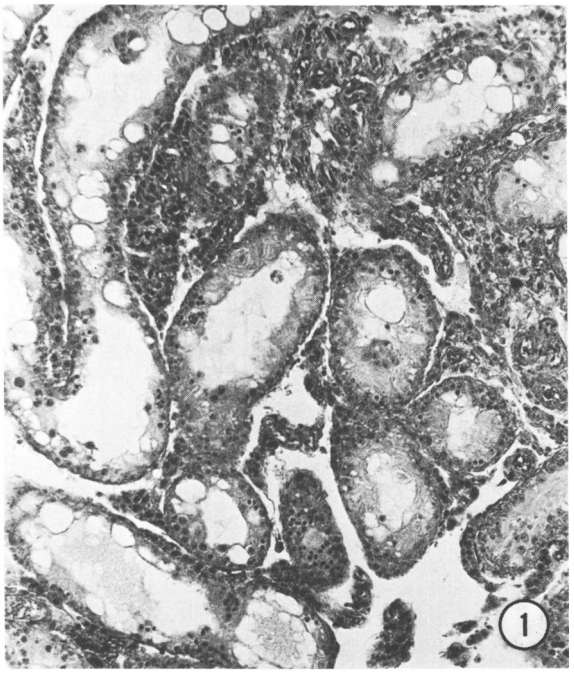

FIG. 1. Seminiferous tubules of the testis of a 24-month-old BALB/c control mouse. Note the numerous large vacuoles. ×100.

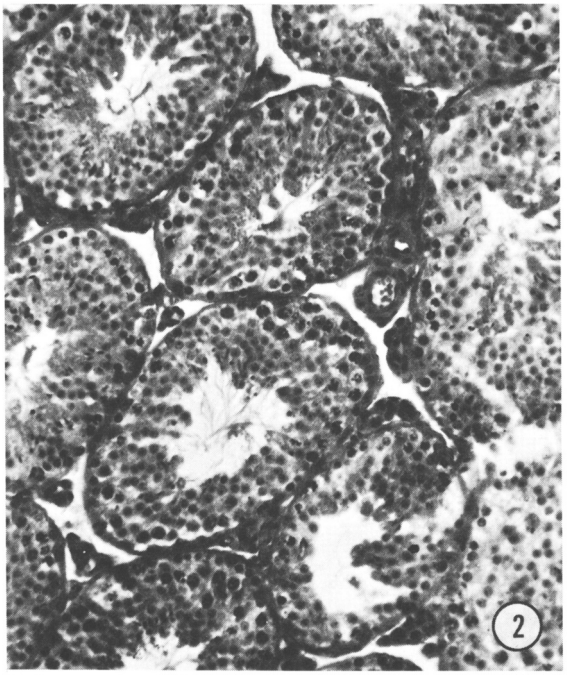

FIG. 2. Testis from a 24-month-old BALB/cfC3H control mouse. Note normal spermatogenesis in seminiferous tubules. ×100.

did not have an effect on spermatogenesis in either substrain with the exception of 1 BALB/c mice—one control and two neonatally treated with 20 μg of estradiol + which showed no spermatogenesis (Figs. 3 and 4). Spermatogenesis was completely suppressed in 6 of 7 BALB/c mice and in 13 of 17 BALB/cfC3H mice neonatally treated with 20 μg of estradiol. In 4 of the BALB/c and 9 of the BALB/cfC3H estrogenized mice, at least one testis was completely replaced by fibrous tissue (Fig. 5). Similar testicular degeneration was also observed in some mice treated with estradiol/progesterone combinations. In animals where tubular structures were present, the interstitial cells appeared normal. In three BALB/c mice—one control and two neonatally treated with 20 μg of estradiol + 100 μg of progesterone—interstitial cell hyperplasia occurred (Fig. 6).

Epididymis. Epididymal cysts occurred in 47% of all hormone-treated mice (Fig. 7). Epididymides of all control mice were normal, with the exception of one BALB/cfC3H mouse where the left epididymis was enlarged with cystic areas. The majority of mice treated with estradiol or estradiol/progesterone which did not exhibit epididymal cysts showed epididymal degeneration. One of 12 BALB/cfC3H mice and five of 22 BALB/c mice neonatally treated with progesterone had epididymal cysts.

Seminal vesicle. Control mice and mice neonatally treated with progesterone had normal seminal vesicles. Mice treated neonatally with 20 μg of estradiol had seminal vesicles in which the epithelium showed signs of atrophy and the lumen was narrow and unbranched (Fig. 8); half of the animals so treated showed areas of metaplasia in the epithelium. The majority of mice neonatally treated with either 5 μg of estradiol or estradiol/progesterone combinations displayed atrophic epithelium. All hormone-treated mice with an atrophic epithelium also showed lymphocytic infiltration.

Prostate, coagulating gland, and ductus deferens. In all mice treated with 20 μg of estradiol the alveolar lumina of both the dorsal and ventral prostates were collapsed

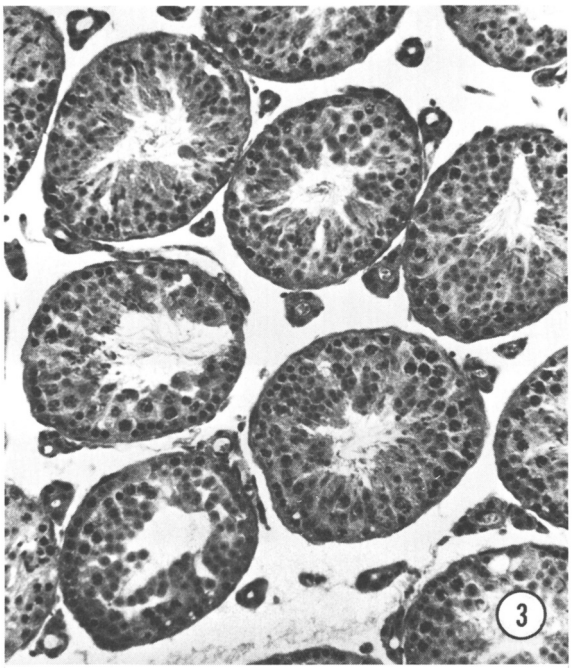

FIG. 3. Seminiferous tubules of the testis of a 24-month-old BALB/c mouse neonatally treated with 100 μg of progesterone. ×100.

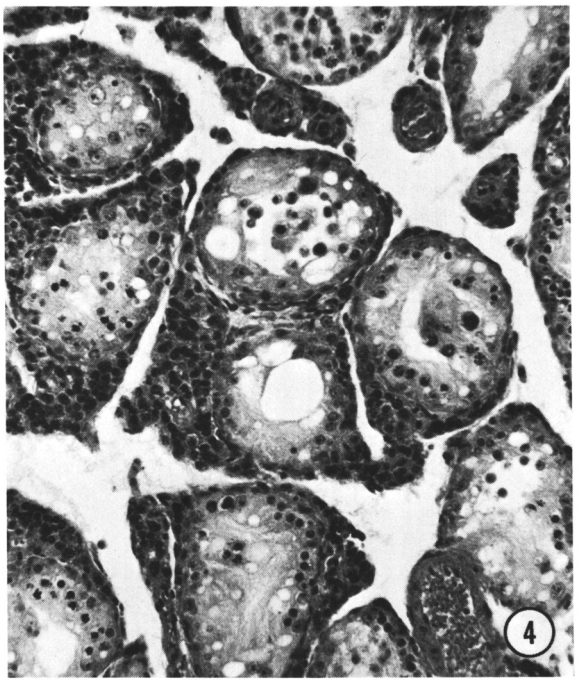

FIG. 4. Seminiferous tubules of the testis of a 24-month-old BALB/c mouse neonatally treated with 100 μg of progesterone. Note the presence of vacuoles. $\times$100.

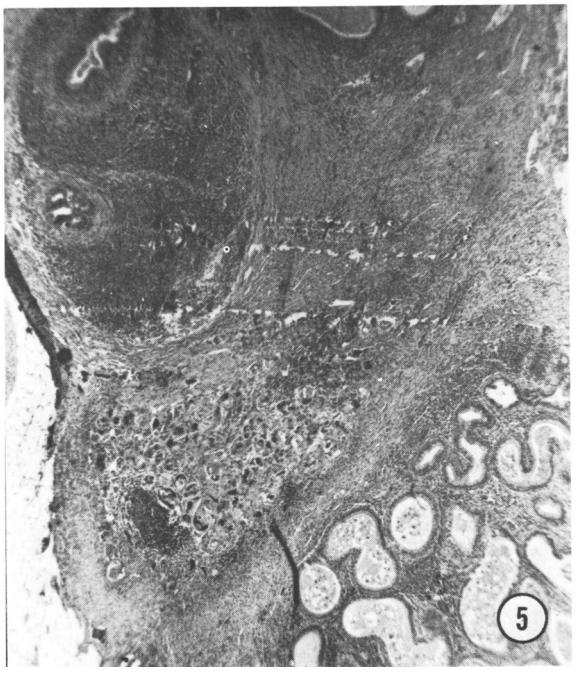

FIG. 5. Atrophic testis from a 20-month-old BALB/c mouse treated neonatally with 20 μg of 17β-estradiol. Note the lack of seminiferous tubules and interstitial cells and the presence of fibrous tissue. $\times$100.

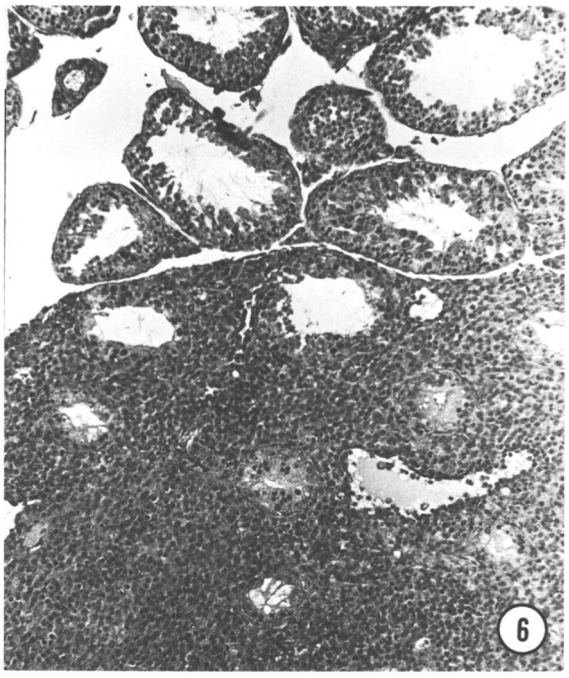

FIG. 6. Testis from a 24-month-old BALB/cfC3H mouse treated neonatally with 20 μg of 17β-estradiol + 100 μg of progesterone. Note the presence of interstitial cell hyperplasia. $\times$100.

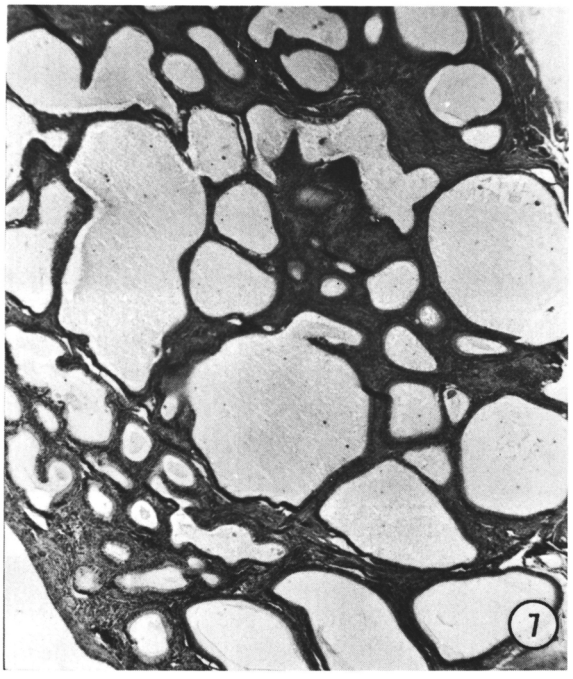

FIG. 7. Epididymis from a 19-month-old BALB/c male mouse treated neonatally with 5 μg of 17β-estradiol. Note the dilated tubules and small amount of connective tissue. $\times$40.

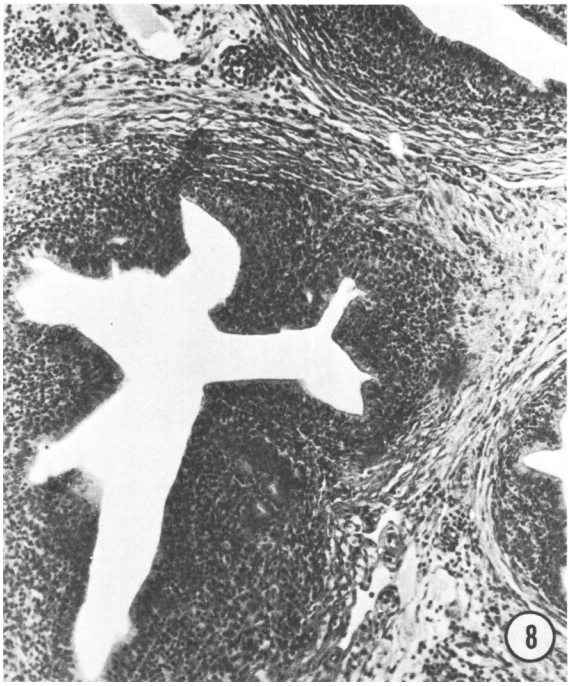

FIG. 8. Seminal vesicle from a 23-month-old BALB/cfC3H mouse treated neonatally with 20 μg of 17β-estradiol. Note the atrophic epithelium. $\times$100.

with areas of heavy lymphocytic infiltration. The majority of the other hormone-treated groups had atrophic prostatic tissue, with the exception of those mice treated with progesterone alone. Only 5 of 22 neonatally progesterone-treated BALB/c mice showed atrophic prostatic tissue, and no similarly treated BALB/cfC3H had such abnormal features. The coagulating gland and the ductus deferens of neonatally hormone-treated animals were degenerated only in those mice whose other sex accessories were also atrophic. The sex accessories of all control mice were normal.

Adrenal glands. The adrenal glands of both control and hormone-treated mice exhibited nodular hyperplasia, ceroid deposition and lymphocytic infiltration. However, these histological features were more extensive in hormone-treated mice.

Discussion. The long-term effects of neonatal exposure of female rodents to steroids and to synthetic estrogens have been well documented (2, 3). However, the long-term effects of such exposures on male rodents at advanced ages have not been so well studied. Daily injections of estrogen for 10 to 15 days from the day of birth cause testicular damage and the permanent arrest of spermatogenesis in mice at later ages (11, 12); even a single injection of estrogen caused testicular damage (18). Perinatally administered DES also causes abnormalities in male sex accessories (10, 15). Although no evidence of neoplastic lesions has been reported in the male mouse genital tract, metaplastic changes occur (10, 15). Squamous metaplasia occurs in the anterior prostate and ejaculatory ducts and papillary hyperplasia of periurethral regions of these structures in neonatally castrated rats receiving DES (14, 18). The severity of these lesions increases with age (20).

The results reported herein on male mice at about 2 years of age indicate that neonatal steroid administration does not cause neoplastic changes but may lead to sterility and complete atrophy of the testis. The observed effects of estrogen and estrogen/progesterone on the genital tract of male mice may be explained, in part, by effects of estrogen at the hypothalamo-

hypophysial level. The effect of neonatal estrogen injections on the testis of mice can be counteracted by simultaneous injections of androgen or gonadotropins (20, 21), leading to the conclusion that the absence of spermatogenesis in the testis of neonatally estrogenized mice was due to insufficient amounts of FSH and LH. In addition, neonatal estrogen treatment caused destructive changes in the plasma membrane of the germ cells and in myoid cells of the connective tissue of the testes of mice by 10 to 20 days of age (22). However, the continued atrophy of the germ cells is probably attributable to the suppressed secretion of gonadotropins, with the degenerated germ cells being phagocytized by Sertoli cells (23, 24). In male rats, neonatal estrogen treatment significantly decreased the number of gonadotropes in the pituitary when compared with controls (6). Similar results were shown to occur in neonatally estrogenized male and female mice (10, 24).

The failure of neonatal progesterone treatment to induce severe abnormalities in the male genital tract in the majority of animals treated indicates the absence of a direct effect on male reproductive structures (26). The cases where progesterone alone did have an effect may have resulted from the conversion of progesterone to testosterone by extragonadal tissue (27). The action of progesterone given concurrently with estrogen was probably more synergistic than antagonistic, with regard to the hypothalamus, as has been demonstrated when the two steroids are given concurrently (28). Recently, Tapanainen *et al.* (29) has shown that neonatal exposure of rats to high progesterone treatment resulted in an increase in the number of Leydig cells. However, these changes were observed at 1 to 3 weeks of age.

Dunn and Green (9) reported that in mice neonatal administration of DES caused destructive changes in the epididymis including degenerated or cystic areas. Both Mori (10) and McLachlan *et al.* (14) noted that perinatal estrogen treatment resulted only in epididymal cysts. In the present investigation, neonatal treatment with either estrogen or estrogen/progesterone brought about both epididymal degeneration and epididymal cysts, in agreement with the findings of Dunn and Green (9). The variable effects of neonatal steroid treatment may depend on whether the neonatal steroid treatment permanently altered the epithelial lining of the epididymis (11, 30).

In female mice neonatally treated with sex steroids and examined at approximately 2 years of age, severe genital tract lesions developed, two of which could be described as frank tumors (31). However, no comparable lesions were found in the 2-year-old male mice neonatally treated with sex steroids. The absence of tumorous lesions may be due to the antagonistic action of testicular androgen during the perinatal period following birth (20). Antagonism between androgen and estrogen has been demonstrated in the reproductive accessory glands of adult male mammals (27, 32). In addition, Arai (8) found that neonatal castration of neonatally estrogen-treated rats increased the incidence of neonatal estrogen-induced metaplasia to 100% and that neonatal exogenous androgen treatment reversed it.

The data reported herein from the neonatal mouse model are in accord with previously reported long-term studies and raise the possibility that antenatal exposure of the human male to DES and female sex hormones can result in testicular pathology, infertility, and other effects upon the genital tract. Recently, Bibbo *et al.* (4) have described abnormalities of the reproductive tract of human males exposed to DES *in utero*. The abnormalities included epididymal cysts in four of 42 males exposed to DES, along with two instances of hypoplastic penis, two of hypoplastic testis, and one of a testicular mass. Gill *et al.* (5) reported a higher incidence of abnormalities of the genital tract in DES-exposed males than in control males. A suggested relation between maternal progestins and human hypospadias has recently been suggested (32).

The author wishes to thank Professor Howard A. Bern for his review of the manuscript, Ms. Aniko Mos for microtechnical assistance, Mr. Jimmy Louie and Ms. Karen T. Mills for general help, Dr. Julian Hoffman for statistical assistance, and Mr. John

Underhill for the photography. This work was aided by NIH Grants CA-05388 and CA-09041 to H. A. Bern and a National Fellowship to L. A. Jones.

1. Herbst, A. (ed.), "Intrauterine Exposure to Diethylstilbestrol in the Human," American College of Obstetricians and Gynecologists (1978).
2. Rice, J. M. (ed.), "Perinatal Carcinogenesis," Nat. Cancer Inst. Monograph No. 51 (1979).
3. Bern, H. A., Jones, L. A., Mills, K. T., Kohrman, A., and Mori, T., J. Toxicol. Environ. Health, Suppl. 1, 103 (1976).
4. Bibbo, M., Al-Naquub, M., Baccarini, I., Gill, W., Newton, M., Sleeper, K. M., Sonek, M., and Wied, G. L., J. Reprod. Med. **15**, 29 (1975).
5. Gill, W. B., Schumacher, G. F. B., and Bibbo, M., J. Reprod. Med. **16**, 147 (1976).
6. Arai, Y., Endocrinol. Japon. **11**, 153 (1964).
7. Arai, Y., Experientia **24**, 180 (1968).
8. Arai, Y., Endocrinology **86**, 918 (1970).
9. Dunn, T. B., and Green, A. W., J. Nat. Cancer Inst. **31**, 424 (1963).
10. Mori, T., J. Fac. Sci., Univ. Tokyo IV **11**, 243 (1967).
11. Takasugi, N., Endocrinol. Japon. **17**, 277 (1970).
12. Warner, M. R., Warner, R. L., and Clinton, C. W., Biol. Reprod. **20**, 310 (1979).
13. Arai, Y., and Suzuki, Y., *in* "Proceedings of 10th International Congress on Anatomy," p. 54 (1975).
14. McLachlan, J. A., Newbold, R. R., and Bullock, B., Science **190**, 991 (1975).
15. Rustia, M., and Shubik, P., Cancer Lett. **1**, 139 (1976).
16. Arai, Y., Chen, C. Y., and Nishizuka, Y., Gann **69**, 861 (1978).
17. Kachi, T., and Kachi, H., Acta Anat. Nippon. **48**, 202 (1972).
18. Arai, Y., "Proceedings of the Seminar on Long-Term Effects of Perinatal Hormone Adminstration," p. 60 (1972).
19. Arai, Y., Suzuki, Y., and Nishizuka, Y., Virchows Arch. Pathol. Anat. Histol, **376**, 21 (1977).
20. Takasugi, N., and Furukawa, M., Endocrinol. Japon **19**, 417 (1972).
21. Takasugi, N., and Mitsuhashi, Y., Endocrinol. Japon. **19**, 423 (1972).
22. Ohta, Y., and Takasugi, N., Endocrinol. Japon. **21**, 180 (1974).
23. Bawa, S. R., J. Ultrastruct. Res. **9**, 459 (1963).
24. Kawashima, S., Bern, H. A., Jones, L. A., and Mills, K. T., Endocrinol. Japon. **25**, 341 (1978).
25. Katsh, S., Endocrinology **47**, 370 (1950).
26. Price, D., and Williams-Ashman, H. C., *in* "Sex and Internal Secretions" (W. C. Young, ed.), Vol. 1. Waverly Press, Baltimore (1961).
27. Zarrow, M. S., Philpott, J. E., and Denenberg, V. H., Proc. Soc. Exp. Biol. Med. **128**, 269 (1968).
28. Krose, R., Berkvens, J. M., Loendersloot, H. J., and Ruitenberg, E. J., Zbt. Vet. Med. **A 18**, 717 (1971).
29. Tapanainen, J., Penttinen, J., and Huhtaniemi, Biol. Neonate **36**, 290 (1979).
30. Jones, L. A., and Bern, H. A., Cancer Res. **39**, 2560 (1979).
31. Price, D., Nat. Cancer Inst. Monograph No. 12, p.1 (1963).
32. Aarskog, D., N. Engl. J. Med. **300**, 75 (1979).

Received February 4, 1980. P.S.E.B.M. 1980, Vol. 165.